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(54) **Enzymatically aided soaking process for skins and hides**

(57) An enzymatically aided soaking process for hides and skins uses proteolytic and lipolytic enzymes and surfactants in an aqueous soaking float containing

(A) lipases with optimum activity in the pH range 9 to 11;

(B) proteases effective in the pH range 9 to 11; and

(C) surfactants,
the pH of the soaking float being in the range 9 to 11.

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ENZYMATICALLY AIDED SOAKING PROCESS
FOR SKINS AND HIDES

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The present invention relates to an enzymatically aided soaking process for skins and hides, in particular to a method of soaking salted, dried and fresh hides with enzyme products which contain alkaline lipases.

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At the start of the beam house procedure an important step is the soaking of the hides and skins. The function of the soaking is to cleanse the raw hide of any dirt adhering thereto, to remove preserving salts and other preservatives from the hide, to at least

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partially dissolve any water-soluble protein constituents from the hide and to give the hide the degree of swelling which it had in its native state and which it lost during the course of the preservation process. (See. H. J. Rehm and G. Reed, Biotechnology, Vol. 6b, p. 734 - 735, VCH 1988). Nowadays, the soaking adjuvants used are primarily surface-active and degreasing agents as well as proteolytic enzymes. These remove any adhering dirt and natural grease and so have the effect of accelerating the rehydration and fibre separation process. The soaking process is preferably carried out at pH 9 to 10.

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This known procedure has the advantage that the jump in pH on moving to the subsequent liming process is minimised and bacterial growth is suppressed. In greasy raw hides it has been found that the soaking process and subsequent opening of the hides always presuppose very good degreasing. (See US-A-4344762; DE-A-3312840). Therefore, in addition to special emulsifiers, lipases are also used, as is clear from numerous passages in the literature (see L.H. Posorske, J. Am. Oil. Chem. Soc. 61 (11), 1758 - 60 (1984)). The use of lipases extends primarily to a pH range of 6 to 9. However, the

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industrial use of lipases was limited by the experiences in the detergent sector, for example, which are summarised in the literature as follows: "However, it (i.e. lipase) cannot be applied as a detergent enzyme, because of its instability under alkaline conditions and its expensiveness" (see H.J. Rehm & G. Reed, Biotechnology, Vol. 7a, p. 644, VCH 1987). The known degrading action of proteases on lipases as proteins has always suggested that lipases and proteases could not be used together.

In modern terms the soaking serves not only to cleanse the hide but also to remove any ingredients which might have a harmful effect on subsequent operations, e.g. grease in the hide. The enzymatically aided soaking process of the prior art was not completely satisfactory in a number of respects, even when lipases were used as well. Thus, there was an unfavourable price-performance ratio when lipases were used and, moreover, it was not unusual for flecks to form in the leather, the flecks being caused by streaks of lime. The aim of the invention is therefore to provide soaking methods which will avoid these disadvantages while achieving an equally good soaking action.

It has now been found that the soaking process according to the present invention is well able to solve this problem. The present invention relates to an enzymatically aided method of soaking hides and skins, for example in the salted, dried or fresh state, using proteolytic and lipolytic enzymes as well as surfactants in an aqueous float, using not only proteases with a sufficient level of activity at pH 9 to 11 but also lipases which have a (marked) activity peak at between pH 9 and 11, the pH of the soaking liquor being in the range from 9 to 11, preferably from 9.5 to 11.

Thus, the present invention provides an enzymatically aided soaking process for hides and skins

using proteolytic and lipolytic enzymes and surfactants in an aqueous soaking float, said soaking float comprising

- 5 A) at least one lipase with an optimum activity in the pH range 9 to 11;
- B) at least one protease which is effective in the pH range 9 to 11; and
- 10 C) at least one surfactant, the pH of the soaking float being in the range 9 to 11.

The soaking floats used according to the invention, advantageously having pH values of around 9.5, thus contain, in addition to B) proteases with a sufficient level of activity at a pH between 9 and 11, A) lipases with their optimum activity at pH 9 to 11 and C) surfactants such as emulsifiers and optionally sequestering agents.

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The lipolytic enzymes A)

In accordance with the usual definitions the lipases which are to be used according to the invention are esterases which hydrolyse glycerol esters of fatty acids in aqueous emulsion (E.C. 3.1.1.3.). Preferably, the triglycerides are cleaved in the 1,3-position. By contrast to the lipases of the prior art used for this purpose, in a pH range from 6 to 9, the lipases used according to the invention have a marked optimum activity (e.g. on olive oil or tributyrin) at between pH 9 and 11. Alkaline lipases of this kind were specially developed for the detergent industry. They are microbiological in origin. Potential sources of microorganism strains of this kind, possibly genetically modified, are fungi and bacteria in particular.

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Certain alkaline lipases occur, for example, in *Pseudomonas* strains. *Rhizopus* sp., *Candida* sp. and *Chromobacterium* sp. are also possible sources of lipase. Other important lipase producers are *Geotrichium* sp.,
5 *Aspergillus* spp., *Mucor* sp., *Penicillium* sp.,
Corynebacterium sp., *Propionibacterium* sp. and
Achromobacter sp. Special mention should be made of
Rhizopus arrhizus and *Rh.oryzae*, *Candida cylindracea*,
10 *Chromobacterium viscosum*, *Geotrichium candidum*, *Mucor*
miehi, *Mucor pusillus*, *Penicillium roqueforti* and *P.*
cyclopium, *Corynebacterium acne*, *Propionibacterium*
shermanii, *Achromobacter lipolyticum* and *Aspergillus*
niger, in particular *Aspergillus oryzae*. Certain
15 genetically modified strains have also been found to be
particularly suitable, e.g. an alkaline lipase obtained
by recombination from an *Aspergillus oryzae* strain and
having a marked activity peak at between pH 9 and 11 or
a lipase which is on sale under the name Lipolase™ 30T
(NOVO INDUSTRI A/S, DK 2880 Bagsvaerd, Denmark).

20 In the usual way, the activity of lipases may be
determined using olive oil as substrate and also using
triacetin and tributyrin. [See M. Sémériva et al.,
Biochemistry 10, 2143 (1971); *Pharmaceutical Enzymes*,
edited by R. Ruyssen and A. Lauwers 1978, (FIP) E.
25 Story-Scientia P.V.B.A, Ghent, Belgium].

Where the grease-cleaving activity is expressed in
kilo-lipase units (unit = KLCA) the experiment is
carried out under standard conditions of 40°C, pH 5.5
using tributyrin as substrate (see M. Sémériva,
30 loc.cit.).

For the purposes of the present invention the
lipase activity is expressed in LCA units but measured
at pH 9.5. According to the invention the lipases are
used so that a lipase activity of 100 to 10,000 LCA,
35 preferably 2000 to 4000 LCA per kg of hide is obtained
at pH 9.5 in the soaking and degreasing bath.

The proteolytic enzymes B)

The use of proteases in the soak which develop a sufficient proteolytic activity at pH levels of between 9 and 11, is known per se. (See US Patent No. 4344762).
5 They are neutral (E.C.3.4.24) and particularly alkaline proteases (E.C.3.4.21) (cf. Kirk-Othmer, 3rd Ed. Vol. 9, pp. 199-202, J. Wiley 1980; Ullmann's Encyclopedia of Industrial Chemistry, Vol. A9, pp. 409-414, VCH 1987; L. Keay in "Process Biochemistry 17-21 (1971)), and more
10 preferably:

- alkaline proteases which develop their optimum activity at a pH of around 8 to 13, preferably PH 9-11. These include alkaline bacterial proteases
15 which generally belong to the serine type and alkaline fungal proteases. Particular mention should be made of the proteases obtained from Bacillus strains such as B.subtilis, B.licheniformis, B.firmus, B.alcalophilus,
20 B.polymixa, B.mesentericus, as well as Streptomyces strains such as S.alcalophilus. The most favourable working temperature with alkaline bacterial proteases is generally from 40 to 60°C and for fungal proteases 20 to 40°C.

25 Examples of alkaline fungal proteases include those from Aspergillus strains such as A.oryzae, those from penicillin strains such as P.cyanofulvum or those from Paecilomyces persicinus and the like. The activity of the alkaline fungal proteases is
30 predominantly at a pH in the range from 8.0 to 11.0. As a rule of thumb, an enzyme activity of between 8000 and 10,000 Löhlein-Volhard units [LVU] per gram of enzyme can be assumed.

35 - Neutral proteases with an activity peak in the range from pH 6.0 to 9.0. These include in particular neutral bacterial proteases which generally belong to the metalloenzymes and fungal

proteases, e.g. neutral Bacillus proteases such as B.subtilis, B.natto and B.polymixa, Pseudomonas proteases, Streptomyces proteases, Aspergillus proteases from A. oryzae, A. parasiticus and Penicillium glaucum. Neutral bacterial proteases develop their optimum activity at working temperatures from 20 to 50°C, whereas the most favourable working temperature for neutral fungal proteases is from 35 to 40°C.

The proteolytic activity of the enzymes is conventionally determined by the Anson-Haemoglobin method (M.L. Anson, J. Gen. Physiol, 22, 79 (1939)) or by the Löhlein-Volhard method (modified according to TEGEWA in Leder, 22, 121-126 (1971)). One Löhlein-Volhard unit (LVU) under the test conditions (1 hour, 37°C) corresponds to the quantity of enzyme which will bring about an increase in hydrolysis product in 20 ml of caseine filtrate corresponding to an equivalent of 5.75×10^{-3} ml of 0.1 N NaOH. The protease activity is generally between 1000 and 60,000 LVU per kg of hide, preferably between 2000 and 14,000 LVU per kg of hide.

Depending on the activity, the method according to the invention usually manages with protease quantities of between 0.05 and 0.8% by weight, or as a rule of thumb about 0.1 to 0.25% by weight, based on the weight of the hides and skins used. In the soaking process according to the present invention, additives known per se such as activators, stabilisers and possibly buffer substances may also be added to the soaking float.

The (synthetic) surfactant substances used may be, for example, conventional emulsifiers, particularly those which are suitable for emulsifying grease in water. (See British Patent No. 586540, DE-A-894142, FR-A-899983, FR-A-918523). The non-ionogenic emulsifiers are particularly suitable, for example those

of the following types:

I. Polyglycol derivatives (the names in brackets are commercial products given by way of example)

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- α) fatty acid polyglycols (EMULPHOR \circledast)
- β) fatty alcohol polyglycolether (DEHYDOL \circledast)
- γ) alkylphenolpolyglycolether (EUMULGIN \circledast 286,
FLUIDOL W100 \circledast ,
MARLOPHEN,
IGEPAL \circledast)

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- δ) fatty acid ethanolamide
polyglycolether (C \circledast , FORYL KW \circledast ,
EUMULGIN)

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II. Glycerol derivatives

- α) fatty acid monoglycerides (TEGOMOLS \circledast)
- β) fatty acid polyglycerol esters

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Other anionic emulsifiers, for example of the following types:

III. Sulphates $R - OSO_3Na$

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- α) fatty alcohol sulphates,
primary and secondary EPPOL DL conc. \circledast ,
PERAMIT ML \circledast TEEPOL \circledast
- β) fatty alcohol ether sulphates (TEXAPON Q \circledast)
- γ) monoglyceride sulphates (VEL \circledast)
- δ) sulphatisation products of (LEDEROLINOR DKMS \circledast)
unsaturated oils and fatty acids

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IV. Sulphonates $R SO_3Na$

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- α) alkylbenzenesulphonates (MARLOPON \circledast , MARLON \circledast)
(ABS, TPS)

- β) alkylsulphonate (MERSOLAT ®)
 γ) fatty acid condensation products (IGE PONA ®, IGE PONT ®)
 δ) petroleum sulphonates (included in: GRASSAN B ®)
 5 ε) sulphitisation products of unsaturated fatty oils and fatty acids (CUTISAN BS ®)
 10 ζ) short-chained alkylbenzene-sulphonates, e.g. of cumene, toluene or xylenol

Less preferred are cationic emulsifiers, e.g. of the following type:

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V. Amine salts $R NR_1, R_2 Hx$ (SAPAMIN ®, SOROMIN ®)

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VI. Quaternary ammonium salts $RN \begin{array}{c} R_1 \\ | \\ \oplus R_3 X \ominus \\ | \\ R_2 \end{array}$ (REPELLAT ®)

- α) ammonium salts
 β) pyridinium salts

25 where the group R mentioned hereinbefore represents a long-chained alkyl group having 8 to 24 carbon atoms whilst the groups R_1 , R_2 or R_3 are generally intended to represent short-chained alkyl groups having up to 6 carbon atoms.

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Emulsifiers which may be used according to the present invention have an HLB value (O/W emulsion) of 8 to 18, preferably 9 to 15, more particularly 12 to 15.

(See Ullmanns Encyklopädie der Techn. Chemie, 4th Edition, Vol. 10). Advantageously it is also possible

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to use combinations of emulsifiers, particularly non-ionic and anionic emulsifiers. Special mention should be made of ethoxylated alkylphenols

(alkylphenolpolyglycols) with an ethoxylation level (E.O.) of 4 to 40, preferably with 6.5 - 12 E.O. per mol of nonylphenol, optionally combined with anionic emulsifiers.

5 The content of emulsifiers in the soaking float is generally from 0.1 to 1% by weight, based on the salted or green weight, depending on the type of emulsifier used. Furthermore, component C) of the soaking float may also contain sequestering agents known per se (cf.
10 Kirk-Othmer 3rd Ed. Vol. 5, pp. 343-368, J. Wiley 1979).

 The sequestering agents may be selected from the group comprising the polyphosphates, phosphonates, polycarboxylates, ethylenediamine tetraacetic acid
15 (EDTA); nitrilotriacetic acid and diethylene triaminopentaacetic acid. The content of sequestering agents in the soaking float may be from 0 to 0.5% by weight, preferably from 0.05 to 0.15% by weight.

20 The soaking process

 As has already been mentioned hereinbefore the soaking process in the beam house is used, inter alia, to free the hides from any blood and soil adhering to them and remove the salt from hides which have been
25 preserved with salt.

 It may therefore be advantageous to carry out a so-called dirty soak first. It is generally sufficient to carry out this soak with water at about 30°C for a certain length of time, for example 2 hours. The
30 vessels used may be the soaking vessels normally used for this purpose, e.g. mixer, drum, tanning machine or paddle. (See F. Stather in Gerbereichemie und Gerbereitechnologie, 4th Edition, Akademie-Verlag, Berlin 1967). A float length of 200% may be taken as a
35 guide.

 It is generally advantageous to aid the soaking operation by mechanical agitation. The soaking liquor

from the dirty soak may expediently be discarded. The pH of the soaking float may be adjusted to between 9 and 11 by the addition of alkalis, e.g. basic sodium or potassium compounds such as sodium hydroxide solution, potassium hydroxide solution, soda, potash and the like.

Conveniently components A), B) and optionally the sequestering agents of component C) in the quantities specified hereinbefore are applied together with the usually liquid surfactants (in the form of detergents). However, it is also possible to use all the components in the form of aqueous or non-aqueous liquid formulations. In these formulations the sequestering agent is present in water-soluble form and the surfactants, preferably of a non-ionic nature, act as stabilisers.

Thus according to another aspect of the present invention there is provided a soaking float for soaking hides and skins, said float being as hereinbefore defined for use in the enzymatically aided soaking process of the present invention.

The soak according to the invention, like the dirty soak, is advantageously carried out in the vessels conventionally used, with agitation. As a guide, when the operation is carried out in the tanning vessel, agitation at about 4 rpm may be used, for example. The temperature of the soak may be $28 \pm 5^{\circ}\text{C}$ as a guide. The soak generally takes some hours, e.g. 3 to 7 hours, whilst 6 hours may be taken as a guide. Conveniently, the soaking liquor is poured away at the end of the soak. After the soak the hides and skins can be further processed in known manner, for example taken for liming (cf. H.J. Rehm and G. Reed, Biotechnology, Vol 6b, 734, VCH 1988). The float length of the soaking float is advantageously from 100 to 300%, based on the total weight of the hides and skins.

The soaking process according to the invention meets the requirements of the art to a particularly high

degree. Even in raw hides with a high natural grease content, e.g. pig skins and sheep skins, an excellent soaking and degreasing action is observed. According to the results obtained hitherto, the degreasing levels are
5 40 to 60% higher than those achieved without the joint use of alkaline lipases. Unexpectedly, the use of alkaline lipases according to component A) reduces the proportion of proteases according to component B) required. If protease is not used, the degreasing
10 action is reduced. If the emulsifiers according to component C) are omitted the degreasing action falls dramatically.

The following Examples serve to illustrate the invention in a non-limiting manner.

Instead of the sequestering agent D mentioned, it is also possible to use hexametaphosphoric acid, tartaric acid, citric acid, gluconic acid, 5-sulfosalicylic acid, nitrilotrimethylenephosphonic acid, ethylenediamine tetramethylenephosphonic acid or hydroxyethylidenediphosphonic acid equally successfully.

2. Examples of industrial application

10 Example 2.1.

10 kg of salted cattle hides Class II, 30/39 kg (weight class 30-39 kg) were subjected to a dirty soak (in a drum) under the following conditions:

15

200.0% water at 26°C. This was left to run for 1 hour and then the float was poured away.

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The hides were subsequently subjected to the main soak and degreasing with the following conditions and components:

150.0% water;

0.3% test product a) to h);

25

0.3% standard emulsifier, consisting of 70% by weight of nonylphenoethoxylate, 8 to 9 mol of ethylene oxide and 30% by weight of the Na-salt of a C₁₂₋₁₈ fatty alcohol ether sulphate with 2 mol ethylene oxide; and

30

0.5% sodium hydroxide solution, 33%, pH 9.5 to 10.5;

Agitation was continued for 6 hours in the drum at 4 rpm; then conventional lime sulphide liming was carried out and the float poured away.

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A sample was taken from the float and the grease content determined. The quality of the soaking effect

was determined in terms of the rapidity of water uptake (rehydration), the degree of fibre separation, the scud purity and the grain distortion of the hides and is rated from 1 (very good) to 6 (unsatisfactory). The results are listed in the following Table.

Example 2.2.

10 kg of salted pig skins were subjected to a dirty soak analogously to Example 2.1.

They were then subjected to a main soak with the following conditions and components:

15 150.0% water at 30°C;
0.3% of test products a)-h);
0.6% of sodium hydroxide solution, 33%, pH 9.5-10.5;
0.3% standard emulsifier (cf. Example 2.1).

20 Agitation was continued for 6 hours and then the float was poured away. The amount of grease in the soaking float was then measured.

25 The quality of the soaking effect was determined in the same way as for Example 2.1 , and the results are listed in the following Table.

30 For both Example 2.1 and Example 2.2 the soaking was followed by conventinal liming and tanning.

TABLE

Test Product	Example	Emulsifier	Fat (g/l) in soaking float	Soaking Effect 1=very good 6=Unsatisfactory
a	2.1	Standard*, 0.3%	3.5	1 - 2
b	2.1	Standard*, 0.3%	2.2	3 - 4
c	2.1	Standard*, 0.3%	2.98	3
d	2.1	Standard*, 0.3%	3.6	1 - 2
e	2.1	Standard*, 0.3%	2.5	2 - 3
f	2.1	Standard*, 0.3%	3.25	2 - 3
g	2.1	Standard*, 0.3%	3.1	3+
h	2.1	Standard*, 0.3%	3.0	3
-	2.1	No emulsifier	1.2	4 - 5
-	2.1	Standard*, 0.3%	2.05	3 - 4
a	2.2	Standard*, 0.3%	14.25	2
b	2.2	Standard*, 0.3%	8.3	3 - 4
c	2.2	Standard*, 0.3%	10.8	3 - 4
-	2.2	Standard*, 0.3%	7.2	4
-	2.2	Standard*, 0.3%	3.1	4 - 5
a	2.2	Dodecylbenzene Sulfonates 0.3%	11.3	3+
a	2.2	Na-laurylsulfate 0.3%	10.8	2 - 3
a	2.2	Mixture	15.5	2 - 3

* = Standard emulsifier from example 2.1

** = Consisting of 70% by weight Nonylphenol with 8-9 mol EO
30% by weight C₈₋₁₈ alkytrimethylammoniumchloride

CLAIMS

1. An enzymatically aided soaking process for hides and
5 skins using proteolytic and lipolytic enzymes and
surfactants in an aqueous soaking float, said soaking
float comprising
 - 10 A) at least one lipase with an optimum activity in the
pH range 9 to 11;
 - B) at least one protease which is effective in the pH
range 9 to 11; and
 - 15 C) at least one surfactant,
the pH of the soaking float being in the range 9 to 11.
2. A process as claimed in claim 1, wherein said
proteases are alkaline proteases with an optimum
20 activity in the pH range of 8 to 13.
3. A process as claimed in either claim 1 or claim 2,
wherein the soaking float also contains sequestering
agents.
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4. An enzymatically aided soaking process for hides
and skins as claimed in claim 1 substantially as herein
described and with reference to the Examples.
- 30 5. A soaking float for soaking hides and skins, said
float comprising
 - A) at least one lipase with an optimum activity in the
pH range 9 to 11;
 - 35 B) at least one protease which is effective in the pH
range 9 to 11; and

c) at least one surfactant,
the pH of the soaking float being in the range 9 to 11.

- 5 6. Hides and skins when treated by a soaking process
as claimed in any of claims 1 to 3.